THE EFFECTS OF BISON MEAT CONSUMPTION ON BLOOD LIPIDS AND SELECTIVE BIOMARKERS RELATED TO CARDIOVASCULAR RISK

by

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ABSTRACT

Elevated serum lipids, blood pressure, and inflammation are related to dietary fat content and can increase the risk for cardiovascular disease. Diets lower in saturated fat have been shown to decrease cardiovascular disease risk. Dietary recommendations to consume leaner meats are often misconstrued as instructions to eliminate or greatly reduce red meat intake instead of consuming leaner red meat. The aim of this study is to investigate and compare the effects of daily consumption of two red meats, bison and beef, as part of a normal diet on cardiovascular risk factors. Twenty-four male and female volunteers (44.3±8.6 years, range 25-59 years), participated in a double-blind cross-over 16-week free living study. Prior to the trial, participants' lipid profiles ranged from normal to mildly hypercholesterolemic (201.8±34.1 mg/dL, total cholesterol range 160-260 mg/dL). Participants were randomly assigned to consume either bison or beef (6 oz for females; 8 oz for males) for 6 days per week for 42 days. Experimental trials were separated by 30 days. Then, participants consumed the other study meat. Overnight fasting blood samples were taken pre and postexperimental trials and analyzed for total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, hs-C-reactive protein, and lipoprotein particle size. Body weights and composition did not change significantly during the trial. There were no significant changes in any of the cardiovascular risk biomarkers: total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and hs-C-reactive protein.

The results of this study suggest that habitual consumption of moderate portions of lean red meat may be accommodated in the diets of individuals with previously normal blood lipid levels without causing untoward effects upon cardiovascular risk factors.

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LIST OF ABBREVIATIONS

AHA .....American Heart Association

BMI..... Body mass index

CLA..... Conjugated linoleic acid

CRP..... C-reactive protein

CVD..... Cardiovascular disease

HDL.....High-density lipoprotein

hs-CRP.....High sensitivity C-reactive protein

IDL.....Intermediate-density lipoprotein

LDL.....Low-density lipoprotein

Lp(a).....Lipoprotein (a)

MUFA.....Monounsaturated fatty acid

NHANES.....National Health and Nutrition Examination Survey

PUFA.....Polyunsaturated fatty acid

SFA.....Saturated fatty acid

VAP.....Vertical Auto Profile

VLDL.....Very low-density lipoprotein

INTRODUCTION

Cardiovascular disease (CVD) is the most common cause of mortality in the United States according to the most recent data (2003) from the Center for Disease Control (1). CVD affected 71.3 million Americans in 2003 (2). This number has increased from approximately 61 million Americans in 2001. The National Health and Nutrition Examination Survey (NHANES) of 1999-2002 estimates that 1 in 3 adults have some form of CVD (1). Several factors affect the potential risk of cardiovascular disease. Although there are many risk factors, diet is considered a primary contributing factor.

The causes of CVD have been the topic of numerous research studies. Cardiovascular disease begins most often in childhood in the form of atherosclerosis. The process of atherosclerosis is caused by an injury or lesion to the inner endothelial wall of an artery. The endothelial injuries can be caused by inflammation, oxidative stress, hypertension, and oxidized LDL cholesterol, among others (3).

Biochemical indicators of CVD risk include inflammation biomarkers such as C-reactive protein (CRP), oxidative stress markers, and blood lipoprotein profiles. The most commonly assessed blood lipids and lipoproteins include total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. High total cholesterol and LDL cholesterol levels correlate with a higher risk of CVD (4). Low levels of HDL

cholesterol and high triglycerides also increase CVD risk. Diet plays a role in blood lipid and lipoprotein profiles. In fact, diets high in saturated fat have been shown to increase blood lipids and lipoproteins such as total cholesterol and LDL cholesterol (5, 6).

The American diet may be at least partially responsible for the number of CVD deaths in the United States. Data suggest that Americans are eating out more often and consuming increased portion sizes equivalent to several servings of meat during one restaurant meal (7). These eating habits increase both total calories as well as total fat content of the diet. In 2000, according to the USDA Economic Research Service, American meat consumption reached an all-time high with annual total meat consumption (red meat, chicken, pork, fish, and lamb) increasing by 57 lbs over the average annual meat consumption in the 1950s. By itself, annual red meat consumption in the US has increased an average of 7 lbs per person since the 1950s (8). In addition to eating more calories and fat, Americans are also increasing their intake of saturated fats, a known dietary risk factor for CVD (5, 6, 9).

Consequently, the American Heart Association (AHA) recommends Americans choose leaner, healthier cuts of meat to decrease heart disease risk and to improve overall health (9). Healthier cuts of meat include leaner meats, such as lean beef, fish, poultry, and game meat (i.e., bison). The American Heart Association dietary guidelines recommend that saturated fat be less than 7% of the total energy intake and that cholesterol intake be kept to less than 300 mg per day to lower risk of CVD (9). Overall, research suggests that a diet low in saturated fat and cholesterol can lower CVD risk by improving the blood lipid and lipoprotein profile (6, 9-11).

Studies have shown that diets low in saturated fat with protein sources that include lean beef, fish, and poultry improve blood lipid profiles. For example, Scott et al. demonstrated in a randomized study that lean beef and chicken consumption (7-8% of caloric intake from saturated fat) significantly decreased total cholesterol and LDL cholesterol levels in hypercholesterolemic men (11). The triglyceride level was steady for both test groups. Beauchesne-Rondeau et al. demonstrated that hypercholesterolemic men consuming an AHA diet (7-10% of the caloric intake from saturated fat) decreased cardiovascular risk factors such as plasma total cholesterol, LDL cholesterol, and triglycerides after they consumed lean beef, fish, and poultry in a cross-over study (10). Mattson and Grundy showed that a diet high in saturated fat increased serum total cholesterol, and LDL cholesterol levels in men with normal triglyceride levels (6). In a separate study, beef fat by itself was shown to increase total cholesterol and LDL cholesterol levels when added to a cholesterol-lowering lean beef diet (5). Consequently, diets high in saturated fat have been shown to increase the atherogenicity of the lipid and lipoprotein profile when compared to diets high in polyunsaturated fats (6).

Diets low in saturated fat have thus been shown to decrease CVD risk (9-11). An alternative red meat that is low in saturated fat, such as game meat, may prove beneficial to the cardiovascular health of Americans. One alternative to conventional feedlot-fed beef is range-fed bison.

Bison meat is becoming popular for its health attributes. Bison meat consumption has increased 17 % since 2004 and has doubled in the past 5 years (12). Health attributes of bison meat may be due to the fact that bison are mainly grass fed and are not exposed

to questionable drugs, chemicals, and hormones. Nutrient analyses suggest bison meat may offer some health advantages compared to beef. A typical cut of bison meat contains slightly less cholesterol and up to 1/3 less fat per 100 g than lean cooked beef (USDA nutrient database, Release 15). Bison meat also has a more favorable fatty acid profile. Bison meat contains increased polyunsaturated fatty acids, omega-3 fatty acids, and conjugated linoleic acids when compared with typical feedlot-fed beef cattle (13-17). Therefore, the consumption of bison meat may result in an improved human blood lipid and lipoprotein profile in comparison with the consumption of feedlot-fed beef (18).

Bison meat contains not only lower total fat, but also less saturated fat. Reduced amounts of saturated fat in a diet also play a role in the reduction of CVD risk (6). Bison meat contains an increased amount of omega-3 fatty acids which play a beneficial role in eicosanoid formation and in lowering vascular and systemic inflammation, which in turn lowers CVD risk (19, 20). In addition, bison meat contains certain omega-6 fatty acids such as conjugated linoleic acid (CLA isomers, 18:2 cis-9, trans-11 and 18:2 cis-12, trans-10), a fatty acid that is found mainly in ruminant sources. Studies have shown that CLA can reduce atherosclerosis, moderate immune response, decrease inflammation, and regulate fat composition, among other benefits (19-25).

The total fat content, fatty acid distribution, and ratios of polyunsaturated fatty acids (PUFA): saturated fatty acids (SFA) and omega-6:omega- 3 (n-6:n-3) fatty acids all affect CVD by potentially influencing cholesterol concentrations (18). Specifically, range-fed bison has an increased PUFA:SFA ratio, omega-3 content, CLA content, and reduced total fat content which should provide for a more favorable blood lipid profile

(6, 18, 25, 26). A comparison of the fatty acid compositions of bison, beef, and poultry adapted from Rule et al. (17) is summarized in Appendix A.

The increased CLA content in bison meat may have another potential benefit for consumers since CLA may possess anti-inflammatory properties. It is widely accepted that inflammation plays a key role in cardiovascular disease (3). Further, CLA is believed to play an anti-inflammatory role in the diet (27). Consequently, dietary CLA such as that found in bison meat may have important implications for chronic disease.

One way to assess systemic inflammation is to measure serum CRP levels. CRP is an acute-phase reactant protein that is produced by the liver in response to the increased levels of pro-inflammatory cytokines during inflammatory events (28). Thus, CRP is considered a good biomarker of inflammation. CRP has been shown to be a relatively stable measurement because it undergoes minimal circadian rhythm variations (3). The Framingham Heart Follow-Up Study has shown that elevated levels of CRP, along with other traditional markers such as blood lipid and lipoprotein profiles are strong indicators of future cardiovascular disease events (28, 29).

There are currently no published clinical trials comparing the effects of bison and feedlot-fed beef consumption on CVD biomarkers. However, limited data exist from a study conducted on a hybrid cross of bison and beef cattle named the Beefalo (25% bison, 75% beef). This 12-week, randomized, single-blind cross-over study compared the blood lipid and lipoprotein responses to the consumption of ground Beefalo and ground beef in 12 hypercholesterolemic men ranging in age from 35 to 80 years old (30). Each participant consumed 8 oz of meat for 5 days per week for a 4-week period. Trials were separated by a 4-week washout period. Beefalo consumption did not alter serum

lipid and lipoprotein levels. However, beef consumption was shown to increase LDL cholesterol levels.

The scientific evidence for the potential health benefits of lower saturated fat consumption as well as the potential health benefits of specific fatty acids, such as CLA, is strong enough to warrant further investigation into the consumption of bison as a healthy alternative to other red meat in the diet. If positive health outcomes are noted, consumption of food products such as range-fed bison may be considered as part of a healthy, balanced diet.

Therefore, the aim of this pilot study was to examine the effects of dietary bison meat consumption on several cardiovascular risk biomarkers. The present study compared the effects of beef and bison meat consumption on blood lipid profiles, lipoprotein profiles, and inflammation biomarkers. Oxidative stress biomarkers were also studied and these data are reported in Chen (31).

METHODS

Research Design

A convenience sample of 24 males and females participated in this 16-week, double-blind, cross-over study. Participants were assigned to one of two isocaloric meat replacement diets, either: 1) range-fed bison meat or 2) feedlot-fed beef, where the only variant was the type of meat consumed.

The participants consumed their first randomly assigned meat 6 days a week for 42 days, followed by a 30-day washout period during which prestudy diets were resumed. After the washout period, participants then received their second assigned meat for the last 42 days of the study. Since both types of meats were consumed in the study, the only unknown during the study was the order in which the meat was consumed. For compliance and convenience purposes, married couples were assigned to the same meat order.

Sample Selection

Study participants were males and females between the ages of 25-59 years old. Smokers, pregnant women, cancer patients, vegetarians, and individuals being treated by medication for hypercholesterolemia or hypertension were excluded from the study. In addition, potential participants were screened for hypercholesterolemia and hypertension prior to study enrollment. Those with total cholesterol levels greater than 230 mg/dl were excluded from the study unless their HDL level was sufficiently high (>60 mg/dL) to

reduce their overall cardiovascular risk estimate to 1% following the National Cholesterol Education Program Standards (4).

Participants with blood pressure greater than 140/90 mm Hg were excluded from the study as well as participants with a personal history of heart disease, stroke, or diabetes. Participants using supplements and other medications were included in the study (after review and approval by the study physician) with the stipulation that medication dosage remain consistent for the duration of the study or that the researchers would be notified of any changes. The University of Utah Institutional Review Board gave approval for the study.

Study Procedures

Study participants were required to eat 6 oz and 8 oz of bison or beef daily, for females and males, respectively, for 6 days of the week, replacing the meat in their regular diet. One day of the week was considered 'free' in which study participants were not required to eat the study meat and could eat their diet of choice. Meat assignments were color-coded and thus blind to both participants and researchers until the end of the study. Study participants received recipes throughout the duration of the study to help provide diet variety and improve compliance. Meat was distributed weekly and participants submitted a meat consumption checklist at each visit to monitor compliance. Additionally, participants were weighed at each visit to ensure that weight remained stable. If participants gained or lost more than 2 lb/week, communication was initiated with the study dietitian.

Participants were provided with 24 oz of ground meat weekly with an additional 24 oz of roast and steak cuts alternated each week. Study meat was provided by the National Buffalo Foundation. Meat samples were randomly chosen, cored, and analyzed from each cut of meat using three meat cores per sample for serum fatty acid profiles by D. Rule and C. Murrieta (University of Wyoming).

Meat fatty acid analysis. Approximately 1 g of previously freeze dried and uniformly homogenized samples were weighed into hexane rinsed, 20x100mm glass tubes with Teflon-lined caps. Each tube received 16 ml of $CHCl_3$:MeOH:H_20 (1:2:0.8; v:v:v) and vortex-mixed for 24 hours. Total lipids from each sample were extracted according to Bligh and Dyer (32). The extracted lipids from each sample were then partitioned for total Fatty Acid Methyl Ester (FAME) analysis (33) and lipid class (neutral and polar) fractionation (34). FAME analyses were generated from total lipid, neutral, and polar fractions by direct-transesterification using 0.2*M* methanolic-KOH according to the procedure of Murrieta et al. (33). Individual fatty acids were separated using gas liquid chromatography (Model 6890, Agilent Technologies, Santa Clara, CA) equipped with a 100m capillary column (SP-2560; Supelco Inc., Belefonte, PA) autosampler, and flame ionization detector.

Data collection occurred four times during the course of the study both pre and post each meat trial. Prior to each sample collection, participants were asked either to refrain from vigorous physical activity for 36 hours or to maintain prestudy physical activity level. Data collection consisted of the following: a fasting blood draw, first void urine sample, height, weight (SECA Model 703 electronic scale), body fat composition

(TANITA Body Composition Analyzer, Model TBF-300A), waist and hip circumference measurements, and collection of 3-day food and activity records.

Blood samples were drawn by a trained phlebotomist following an overnight fast. The samples were centrifuged and divided into two 2 mL and 4 mL aliquots of serum and stored at –80 °F until analysis at the end of the study. The 2 mL blood serum samples were analyzed by Atherotech (Birmingham, AL) for blood lipid biomarkers and for hs-CRP. Blood lipid biomarkers analyzed included: total cholesterol, direct HDL cholesterol, direct LDL cholesterol, direct triglycerides, direct VLDL cholesterol, LDL particle size pattern, Lp(a) cholesterol, IDL cholesterol, HDL-2, HDL-3, and VLDL-3 using Atherotech's trademarked Vertical Auto Profile (VAP) technology, an inverted rate zonal, single vertical spin, density gradient ultracentrifugation. The 4 mL serum sample was analyzed for fatty acid profile (including PUFA, CLA, and omega-3 fatty acids) by D. Rule and C. Murrieta (University of Wyoming).

VAP method analysis. Two density gradient layers were prepared in a centrifuge tube by dispensing a known amount of 1.006 g/mL saline solution and then underlayering with a known amount of serum that was diluted 40-fold with 1.21 g/mL KBr solution. Sixteen centrifuge tubes with two-layer density gradients (1.21 g/mL KBr layer at the bottom containing serum and 1.006 g/mL saline layer at the top) were centrifuged using a vertical rotor (Beckman VTi-65 Vertical Rotor) in an ultracentrifuge (Beckman Coulter Optima XL-100K, Palo Alto, CA) for 45 minutes at 65,000 rpm. After centrifugation, the contents were analyzed for cholesterol using the continuous flow VAP analyzer. The contents, which were drained from the bottom of the tube by puncturing with a needle, were slowly and continuously mixed with a flowing enzymatic reagent specific for

cholesterol in a heated (37 °C) narrow-bore Teflon tubing. A red color developed as the mixture flowed through the tubing proportional to cholesterol concentration, and the absorbance corresponding to the intensity of the red color was monitored with a spectrophotometric detector at 505 nm. Digital data corresponding to the absorbance were simultaneously recorded by a computer using in-house–developed software. The accuracy of this method is routinely compared to beta-quantified split samples at CDC-designated lipoprotein reference laboratories. Correlation coefficients obtained by split comparison with Core Laboratories for Clinical Studies at Washington University range from 0.91-0.99 for total cholesterol, LDL, VLDL, and HDL cholesterol while IDL and Lp(a) coefficients range from 0.77-0.78 (35).

High sensitivity C-reactive protein analysis. The hs-CRP analysis used latex-enhanced turbidimetric immunoassay utilizing CRP Ultra Wide Range Reagent (Equal Diagnostics, Exton, PA) and the Aeroset Chemistry Analyzer (Abbott Diagnostics, Abbott Park, IL).

Serum fatty acid analysis. Two mL of thawed serum were weighed into a hexane rinsed 16x25 mm screw cap tube with Teflon-lined caps. Total lipids were extracted from the serum using 3 mL of $CHCl_3$:MeOH (1:2) and vortex mixed for 24 hours. Total lipids from each sample were extracted according to Bligh and Dyer (32). The extracted lipids from each sample were then partitioned for total FAME analysis (33) using 1 mg 13:0 as the internal standard. Individual fatty acids were separated using gas liquid chromatography (Model 6890, Agilent Technologies, Santa Clara, CA) equipped with a 100m capillary column (SP-2560; Supelco Inc., Belefonte, PA) autosampler, and flame ionization detector. Identification of peaks was accomplished using purified standards

(Nu-check Prep, Elysian, MN; Matreya, Pleasant Gap, PA). Identification of the 18:1 *trans*-10 isomer was putative and based on the position of a peak between peaks identified as 18:1 *trans*-9 and 18:1 *trans*-11 (36).

The first morning void 5 mL urine sample was collected according to Chen (31). Urine was analyzed for 8-hydroxy deoxyguanosine (8-OhdG) and 8-epi-prostaglandin 2 α (8-epi-PG2α) normalized to urine creatinine by Genox (Baltimore, MD). Both tests are biomarkers of DNA damage and oxidative stress (lipid peroxides).

The participants recorded their food and beverage intake and physical activity for the 3 days prior to the data collection periods using a 3-day food and activity record. Participants were instructed to record the amount of food, preparation method, brand names, and time food was eaten in the food record. Food records were analyzed using Food Processor nutritional analysis software (Version 8.3, 2004, ESHA Research). The activity record included activity type and duration.

Statistical Methods, Data Analysis, and Interpretation

The data analysis was based upon the significance of change from initial to posttreatment analyses for both meat consumption groups. Data were assessed for normality and analyzed using paired sample t-tests. For all analyses, the level of significance was set at $P< 0.05$. CRP data were also analyzed using the nonparametric Wilcoxon test due to non-normal distribution. Data analysis was performed using SPSS (Version 15.0, SPSS) statistical software. Outliers were determined and substituted with the data set mean as determined by the SPSS software.

RESULTS

Participants and Diet

All 24 participants completed both meat consumption trials, and all blood and urine samples were collected. Weekly meat consumption checklist forms indicated that participants were highly compliant in consuming the study meat. Participant demographics are included in Table 1. Results were analyzed for a study meat order effect using a repeated measures analysis of variance test. There was no effect found for the order of the bison and beef consumption for this study.

Dietary analysis between pre and post diets demonstrated that the caloric intake was isocaloric for the prebison and postbison consumption trial of the study. The caloric intake postbeef consumption was significantly higher than prebeef consumption ($P<0.05$). Dietary intake values are summarized in Table 2. Although caloric intake was significantly increased postbeef consumption, body weight, body fat composition, and waist:hip measurements did not significantly change during consumption of either meat. The amount of physical activity was not significantly altered during the course of the study. Body measurements and physical activity summary are presented in Table 3.

Evaluation of the macronutrient content found that the percent of caloric intake per day from protein significantly increased during the bison consumption ($P<0.05$) and during the beef consumption ($P<0.01$). Correspondingly, the percent of caloric intake per day from carbohydrates significantly decreased from pre to post during both bison and

Table 1

Participant Demographics

Category (N=24)		n (%)
Mean Age ± SD (years)	44.3±8.6	
Age Range (years)	25-59	
Mean Total Cholesterol± SD (mg/dL)	201.8±34.1	
Cholesterol Range (mg/dL)	160-260	
Mean BMI± SD (kg/m^2)	25.2±4.2	
Mean waist:hip ratio ±SD	0.83±0.07	
Females		8 (33)
Males		16 (67)
Participating in study as:		
Couples		10 (42)
Individuals		14 (58)

Table 2

Mean Daily Dietary Intake

Intake Measurement	Prebison N=24	Postbison N=24	Prebeef N=24	Postbeef N=24
Energy Intake (kcal/day)	2169.8±692.8	2152.8±667.2	2205.9±938.3	2476.3±706.1*
Protein (% kcal)	16.0±4.0	23.2±5.1*	16.8±4.6	19.6±3.5**
Carbohydrate (% kcal)	49.6±7.2	43.4±6.5**	48.3±7.7	40.1±5.8**
Fat (% kcal)	33.1±6.2	32.8±5.5	34.8±7.8	39.5±5.9**
Saturated fat (% kcal)	10.3±3.0	11.0±2.7	11.5±3.7	13.7±2.5**

*Significantly different pre to post, $P<0.05$.

**Significantly different pre to post, $P\leq0.01$.

Table 3

Body Weight, Fat, and Activity Measurements [a]

Body Measures	Prebison	Postbison	Prebeef	Postbeef
Weight (kg)	76.1±18.4	75.8±18.0	75.6±18.1	75.4±18.0
BMI (kg/m^2)	25.3±4.2	25.2±4.1	25.1±4.2	25.1±4.2
Waist:Hip	0.83±0.08	0.84±0.07	0.84±0.07	0.84±0.07
Body Fat (%)	23.5±7.3	23.6±7.8	23.3±8.2	23.5±8.4
Physical Activity (minutes/day)	77.9±105.4	65.4±95.9	70.4±86.8	74.3±87.8

[a] None of the comparisons, pre to post, were statistically significant. N=24.

beef consumption trials ($P<0.01$). Total percent of caloric intake from fat increased during the beef consumption trial of the study ($P<0.01$) while it did not significantly change postbison consumption. Percent of calories from saturated fat increased postbeef consumption ($P<0.01$), while there was no significant change in the saturated fat intake postbison consumption.

Blood Lipids, Inflammation, and Oxidative Stress

There were no statistically significant differences between pre and post analyses for either type of meat consumption trial among the study population when comparing total cholesterol, LDL cholesterol, Lp(a) cholesterol, IDL cholesterol, HDL cholesterol, triglycerides, and hs-CRP serum. Blood lipid results are presented in Table 4.

The only significant differences found among the study population were in oxidative stress biomarkers (31). In the postbison consumption trial, 8-epi-PG2α normalized to creatinine was significantly lower ($P<0.05$). Another oxidative stress biomarker, 8-OHdG normalized to creatinine, was significantly lower in the postbeef diet ($P<0.05$). Inflammation and oxidative stress biomarkers are presented in Table 5.

Serum fatty acid analysis results indicated statistically different mean difference scores for the bison and the beef consumption trials. The difference score postbison consumption (post-pre) indicated that the linoleic acid found in the serum was much lower ($\bar{x}=0.00\pm0.01$), than during the beef consumption ($\bar{x}=0.18\pm0.17$, $P<0.01$) while other minor fatty acids in the serum were increased ($P<0.05$). Serum fatty acid profiles are included in Appendix B.

Table 4

Mean Serum Lipid Levels[a]

Measurement	Prebison (N=24)	Postbison (N=24)	Prebeef (N=24)	Postbeef (N=24)
Total Cholesterol (mg/dL)	197.2±33.5	198.9±29.9	200.1±32.7	197.8±28.0
Triglycerides (mg/dL)	79.8±27.0	88.4±48.1	84.0±41.2	77.6±24.35
HDL cholesterol (mg/dL)	58.9±19.9	57.3±18.9	56.6±17.8	58.3±15.8
LDL cholesterol (mg/dL)	120.5±24.3	122.9±20.6	124.9±24.5	121.6±22.5
Lp(a) cholesterol (mg/dL)	8.2±3.6	7.8±3.4	7.7±3.5	7.6±3.8
IDL cholesterol (mg/dL)	8.2±6.6	8.8±5.4	9.0±4.5	7.8±5.3
VLDL cholesterol (mg/dL)	17.8±4.0	18.9±6.3	17.8±3.5	18.4±4.0

[a]None of the comparisons, pre to post, were statistically significant.

Table 5

Inflammation and Oxidative Stress Biomarkers[a,b]

Biomarker	Prebison	Postbison	Prebeef	Postbeef
High sensitivity C-reactive protein (mg/dL)	1.97±2.44	1.56±2.12	1.47±1.75	1.34±2.01
8-OHdG (Ng/mg creatinine)	0.13±0.06	0.12±0.05	0.16±0.10	0.11±0.06*
8-epi-PG2α (pg/mL creatinine)	31.22±26.06	22.0±12.5	38.3±37.8	23.8±21.6*

*Significantly different pre to post, $P<0.05$.

[a]Oxidative stress data (8-OHdG and 8-epi-PG2α) from Chen (31).

[b]N=24 for all categories except CRP for pre- and postbison, N=22.

Lipid Sizing and Classification

Lipid classification and sizing analysis results yielded no significant difference pre- to postconsumption of either study meat for HDL-2 cholesterol, the most protective HDL cholesterol subclass. VLDL-3, a small remnant LDL cholesterol, did not statistically change nor did LDL particle size decrease during the study for either bison or beef. However, HDL-3 cholesterol, which is the smaller, less protective HDL cholesterol particle, was decreased significantly after bison consumption ($P<0.05$). Lipid sizing results are included in Table 6.

Age-Related Differences

Some statistical differences were also noted when comparing study results and participants' age. Participants younger than age 45 demonstrated a statistically significant lower LDL cholesterol ($\bar{x}=127.8\pm24.1$ and $\bar{x}=116.5\pm19.2$, pre to post, respectively) upon completion of the beef consumption trial ($P<0.05$).

Statistical differences were also found related to age and body measurements. For those participants younger than age 45, waist:hip ratio mean difference scores were significantly lower postbeef consumption ($\bar{x}=-0.02\pm0.02$, $P<0.05$). The corresponding difference score was not statistically different postbison consumption. Waist:hip ratio means were significantly different postbeef and postbison in those participants younger than 45 years. However, there were no mean differences in the older age group.

Markers of oxidative stress were also affected by participant age (31). For those participants age 45 and older, levels of 8 epi-PG2α normalized to creatinine were significantly lower postbeef consumption ($P<0.01$) while there was no statistical

Table 6

Lipid Sizing Results

Lipid Biomarker	Prebison (N=24)	Postbison (N=24)	Prebeef (N=24)	Postbeef (N=24)
HDL-2 (mg/dL)	14.9±8.1	14.9±8.3	14.6±7.9	14.7±7.2
HDL-3 (mg/dL)	44.1±12.0	42.4±11.0*	42.0±10.6	43.6±9.1
LDL Pattern Size	2.2±0.7	2.3±0.7	2.2±0.8	2.3±0.7
VLDL-3 (mg/dL)	17.8±4.0	18.9±6.3	18.4±3.5	17.8±4.0

*Significantly different pre to post, $P<0.05$.

difference postbison consumption. For those younger than 45, there was no difference in oxidative stress biomarker 8 epi-PG2α after consumption of either meat. However, both age groups benefited from statistically lower 8-OHdG normalized to creatinine levels postbeef diet ($P<0.05$). A summary of age-related results is presented in Table 7.

Gender-Related Differences

Differences in lipid and oxidative stress biomarkers were also noted between genders as summarized in Table 8. Postbeef consumption, females had significantly lower total cholesterol and LDL-cholesterol levels when compared to initial levels ($P<0.05$). HDL cholesterol levels increased for males pre to postbeef consumption ($P<0.05$). According to Chen (31), both oxidative stress biomarkers, 8-OHdG ($P<0.05$) and 8 epi-PG2α ($P<0.01$), were significantly lower postbeef consumption for the males. Females did not exhibit any significantly different oxidative stress biomarkers pre to postbeef consumption. There were no significant biomarker changes for males or females pre- to postbison consumption.

Dietary Meat Analysis

The results of the study meat fatty acid composition analysis for all three cuts of study meat for bison and beef (D. Rule and C. Murrieta, University of Wyoming) are summarized in Table 9. Complete results are included in Appendix C. The overall fat amount was higher in all three cuts of the beef than in the bison meat. Analysis indicated that the omega-3 fatty acid percentage was higher in the bison than in the beef. PUFA and the subsequent PUFA:SFA ratio was greater in the bison cuts of roast and steak than

Table 7

Age-Related Results [a]

Measurement	Prebison	Postbison	Prebeef	Postbeef
Total Cholesterol (mg/dL)				
<45	200.8±36.3	199.6±37.0	203.5±35.6	191.6±25.9
≥45	194.6±32.5	198.4±25.1	197.7±31.6	202.2±29.5
LDL cholesterol (mg/dL)				
<45	121.3±23.6	121.9±22.6	127.8±24.1	116.5±19.2*
≥45	119.9±25.6	123.6±19.8	122.8±25.4	125.2±24.6
HDL cholesterol (mg/dL)				
<45	61.4±23.1	60.2±23.6	57.5±19.9	56.9±17.3
≥45	57.2±17.8	55.1±15.3	56.1±6.9	59.3±15.2
Triglycerides (mg/dL)				
<45	77.4±29.6	81.1±29.0	73.117.0	79.4±18.7
≥45	77.6±22.4	81.2±37.4	79.524.4	74.7±28.8
hs-CRP (mg/dL)				
<45	1.79±1.63	1.37±1.31	1.17±1.20	0.91±0.71
≥45	1.49±2.37	1.88±2.67	1.69±2.67	1.64±2.55
8-OHdG (Ng/mg creatinine)				
<45	0.11±0.03	0.08±0.03	0.12±0.05	0.09±0.04*
≥45	0.14±0.07	0.14±0.05	0.17±0.08	0.12±0.07*
8-epi-PG2α (pg/mL creatinine)				
<45	17.00±10.76	16.59±8.70	17.06±7.13	13.78±4.88
≥45	34.35±21.04	24.58±12.82	43.04±31.33	29.87±26.45**
Body Weight (kg)				
<45	72.8±16.7	72.6±16.4	72.9±17.0	73.1±16.8
≥45	78.5±19.8	78.1±19.4	77.5±19.3	77.1±19.2
Waist:hip ratio				
<45	0.82±0.07	0.83±0.06*	0.84±0.06	0.82±0.07*
≥45	0.84±0.08	0.84±0.08	0.84±0.08	0.84±0.07

*Significantly different pre to post, $P<0.05$.

**Significantly different pre to post, $P<0.01$.

[a]Oxidative stress data from Chen (31). Age <45 years, N=10, for ≥ 45 years, N=14.

Table 8

Gender Summary [a]

Measurement	Prebison	Postbison	Prebeef	Postbeef
Total Cholesterol (mg/dL)				
Male	188.5±30.5	191.6±27.1	189.9±29.3	194.7±29.6
Female	214.5±34.3	213.5±31.7	220.5±30.9	204.0±25.1*
LDL cholesterol (mg/dL)				
Male	120.8±25.5	122.9±21.1	122.7±24.5	124.6±24.5
Female	120.023.2	122.8±20.8	129.3±25.5	115.5±17.8*
HDL cholesterol (mg/dL)				
Male	48.9±10.2	47.9±10.9	47.6±12.1	51.6±11.7*
Female	79.1±19.2	76.0±17.8	74.6±12.9	71.8±14.6
Triglycerides (mg/dL)				
Male	86.9±26.3	92.4±35.3	84.3±21.6	78.8±25.2
Female	65.6±24.0	60.0±13.9	62.5±11.7	75.3±24.0
hs-CRP (mg/dL)				
Male	1.71±231	1.79±2.44	1.68±1.98	1.62±2.40
Female	1.45±1.68	1.10±1.32	1.08±1.20	0.78±0.61
8-OHdG (Ng/mg creatinine)				
Male	0.13±0.06	0.11±0.06	0.15±0.06	0.10±0.04*
Female	0.12±0.06	0.12±0.05	0.15±0.09	0.12±0.08
8-epi-PG2α (pg/mL creatinine)				
Male	32.86±20.94	22.56±12.83	34.61±21.89	21.09±13.94**
Female	15.23±6.04	20.81±12.43	28.91±39.84	29.02±32.66

*Significantly different pre to post, $P<0.05$.

**Significantly different pre to post, $P<0.01$.

[a]Oxidative stress data (8-OHdG and 8-epi-PG2α) from Chen (31). For males, N=16, for females, N=8.

Table 9

Study Meat Lipid Composition, Fatty Acid Weight %[a]

Fatty Acid	Bison Roast	Beef Roast	Bison Steak	Beef Steak	Bison Burger	Beef Burger
Total Fat	1.57±0.33	5.35±2.83	1.84±0.08	4.92±1.35	8.58±0.16	17.04±1.23
CLA (*c*-9, *t*-11)	0.38±0.13	0.31±0.12	0.30±0.07	0.32±0.07	0.30±0.05	0.44±0.01
n3	0.65±0.14	0.16±0.03	0.80±0.14	0.15±0.02	0.32±0.06	0.23±0.01
n6	6.08±2.69	3.21±0.96	9.91±0.91	3.31±0.22	2.26±0.23	1.77±1.24
n6:n3	9.36±0.49	20.05±0.34	12.38±0.20	22.09±0.17	7.07±0.22	7.68±0.03
SFA	41.11±2.94	43.08±2.38	41.19±1.33	46.63±0.90	49.84±0.65	42.69±0.17
MUFA	50.03±5.87	50.73±3.85	45.34±0.91	47.25±0.85	43.60±0.43	50.34±0.52
PUFA	7.11±2.69	3.68±0.97	11.01±0.93	3.78±0.23	2.88±0.24	2.43±0.03
PUFA: SFA	0.17±0.39	0.09±0.27	0.27±0.09	0.08±0.06	0.06±0.09	0.06±0.01
C-14+ C-16	17.25±2.17	28.85±2.15	17.92±0.41	29.10±0.88	19.62±0.43	27.21±0.12

[a]Each meat sample was composed of three cored subsamples from three meat packages chosen at random, combined, and extracted for analysis.

in the beef cuts of roast and steak. Of note, the ground burger for both bison and beef had similar PUFA:SFA ratios (0.06±0.09 for bison and 0.06±0.01 for beef) and n6:n3 ratios (7.07±0.22 for bison and 7.68±0.03 for beef). Both types of meat also had comparable percentages of CLA with the exception of the beef burger, which had a significantly higher amount of CLA than the ground bison burger.

DISCUSSION

The results demonstrate that moderate consumption of lean red meat (6-8 oz daily 6 days a week for 6 weeks) does not significantly increase cardiovascular risk biomarkers as also demonstrated in other studies (5, 10, 11). Comparisons of red meat intake between participants fed range-fed (feedlot-finished) bison and feedlot-fed beef suggest that there was not a significant difference in overall blood lipid profiles for this pilot study.

Lipid particle sizing gave insight to the overall cardiovascular changes associated with the study diet. Results demonstrated that while the total HDL cholesterol was not significantly lowered postbison consumption, there was a significant decrease in the smaller, less protective HDL-2 cholesterol but not in the more protective, HDL-3 cholesterol. LDL pattern size did not decrease for either of the study meats. Thus, although these results are difficult to interpret, since LDL particle size did not become smaller and overall HDL cholesterol was not significantly different, the particle sizing analysis demonstrates that the lipid profile did not become more atherogenic. These results demonstrate how particle sizing can be a potentially useful tool to analyze lipid characteristics in future studies.

There were age and gender-related differences evident in the beef trial. Study participants younger than 45 years old demonstrated significant decreases in LDL

cholesterol and waist:hip ratios after beef consumption. These results suggest that moderate beef consumption may not have a negative health impact on the younger population. The lack of impact may be due to several factors: younger people may be better able to control cholesterol levels in the body (37), study conditions may have caused this population to eat out less than normal, or the controlled meat portions may have actually been lower than the self-reported meat intake for the regular diet.

Gender differences may explain several study outcomes. Females, but not males, exhibited significantly lower total cholesterol values and LDL cholesterol following beef consumption. However, males exhibited significantly increased HDL levels and lowered oxidative stress biomarkers following beef consumption. The increase in HDL cholesterol for males postbeef consumption may be due to the significantly increased intake of total fats during this trial. Studies show that low-fat diets can decrease HDL cholesterol (6, 10, 11, 38) it may follow that increased fat content in the diet may also affect cholesterol levels (5). This mechanism could be interpreted as an adaptive response to fat in the diet permitting the consumption of higher levels of dietary fat without elevating blood total cholesterol. Additionally, females already had significantly higher baseline HDL cholesterol levels. As a negative risk factor for cardiovascular disease, higher HDL cholesterol levels may have moderated the overall study results. The Fels Longitudinal Study demonstrated that age was a significant predictor of increased LDL cholesterol and total cholesterol for males (39) while females were less likely to be affected.

Surprisingly, there were no statistically significant pre- and postlipid values for the bison as there were for the beef. These results were somewhat unexpected based

upon the differences in the total fat content of the meats consumed. Analysis of the study meat (refer to Table 9) suggests that the fatty acid composition of the two ground meats had similar PUFA:SFA and n6:n3 ratios and similar saturated fat content contributing to the lack of effect of the bison meat. The bison burger PUFA:SFA ratio (0.06±0.09) was nearly identical to that of the beef burger (0.06±0.01). The bison burger contained 49.8 wt% SFA and the beef burger contained 42.7 wt% SFA. The other cuts of meat used in the study, both the steak and the roast, contained a more favorable fatty acid composition in the bison meat than in the beef. However, the study design alternated the steak and roast each week while the burger was consumed every week. The weekly consumption of the ground burger may have had an effect in the outcome of the lipid biomarkers since it may have resulted in too similar of a PUFA:SFA ratio between the two meats. Fatty acid composition of the meat intake has been shown to have an effect on lipid and lipoprotein profiles (40) and the lack of differences in lipid outcome between the bison and beef corroborate the lack of overall difference in PUFA:SFA intake.

CRP as an inflammation marker is widely accepted in current research (3, 28, 29). However, this trial found there was no significant difference in CRP levels for the different meat consumption trials. One reason that significant differences in CRP levels may not have been evident was that the meat consumption trials were isocaloric. Therefore, weights remained stable throughout the study. Previous studies have demonstrated that CRP levels are not influenced by dietary fat consumption without a parallel change in body weight (41). By maintaining weight stability during this study, any differences in CRP levels by dietary fat may have been masked.

Although the purpose of this study was to replace meat normally consumed with controlled portions of bison or beef, the amount of calories from protein and overall energy intake, as analyzed from the 3-day food records, was statistically greater for the beef trial. For the bison, overall energy intake was not different, although protein intake was significantly increased. However, there was no difference in the amount of exercise, weight gain, % body fat, or other body measurements between the pre and post time periods. The total fat mean intakes from both bison (pre $\bar{x}$ =33.1%±6.2 and post $\bar{x}$ =32.8%±5.5) and beef (pre $\bar{x}$ =34.8%±7.8 and post $\bar{x}$ =39.5%±5.9) diets were greater than AHA recommendation that less than 30% of calories comes from total fat. AHA recommendations that saturated fat comprise less than 7% of caloric intake was also exceeded during the bison (pre $\bar{x}$ =10.3±3.0 and post $\bar{x}$ =11.0±2.7) and beef (pre $\bar{x}$ =11.5±3.7 and post $\bar{x}$ =13.7±2.5) diets. AHA recommends these intake guidelines to prevent cardiovascular disease. Saturated fat and total fat intake increased pre to post for both the beef and the bison without significant effects on cardiovascular risk biomarkers. These results suggest that there may be a higher upper level of acceptable fat intake than previously assumed in the population. Since this was a relatively short-term study (6 weeks per meat), future long-term studies are needed.

Limitations of this study include, as with any free-living study, incomplete control over participants' diets. In some cases, study meat was eaten in addition to several other meat sources daily. This additional meat consumption may have affected the results of this study. The extent of additional meat consumption throughout the study is unknown since food records were only taken 3 days prior to each blood draw. During the study, checklists were submitted weekly to ensure that the study meat was consumed,

but there was no measure included for additional meats. In some instances, participants did not eat the study meat the day before the blood draw or ate study meat in addition to other meat including chicken, burgers, and shrimp. For future studies, more definitive preblood draw guidelines should be set to ensure that the study results are not compromised. Also, future studies may consider allowing participants to consume other meats only on the 'free day' to prevent skewing the results. Since some participants ate additional meat, diet records suggest that 6-8 oz of meat daily was not an accurate amount for replacement. Future studies should allow participants to replace dietary meat completely with the study meat.

The results of this pilot clinical trial provide data useful for planning future studies of this nature pertaining to the relative risk and safety of consuming red meat-based diets. Overall, the results indicate that consumption of lean red meat, whether bison or beef, in moderate amounts can be a larger part of the diet than popularly suggested without increasing cardiovascular disease risk.

APPENDIX A

FATTY ACID COMPARISON DATA

Fatty Acid Weight Percentage Comparisons of Semitendinous Muscle[a]

Fatty Acid	Range-fed bison	Feedlot-fed Beef	Chicken Breast
cis-9, trans-11 CLA	0.39	0.28	0.07
trans-10, cis-12 CLA	0.03	0.01	0.00
SFA	38.1	42.0	34.7
PUFA/SFA	0.52	0.15	0.71
n-3	6.92	0.95	1.19
Cholesterol (mg/100g)	45.8	53.4	59.3

[a] Data adapted from Rule et al., 2002 (17).

APPENDIX B

SERUM FATTY ACID COMPOSITION DATA

Serum Fatty Acid Concentrations Pre and Post Meat Consumption

	Beef			Bison		
Serum Fatty acid	**Pre**	**Post**	**p**	**Pre**	**Post**	p
14:00	0.0082±0.005	0.0135±0.008	0.008	0.0092±0.006	0.0124±0.007	
14:1 c9	0.003±.001	0.0039±.002	0.034	0.0035±0.002	0.0038±0.001	
15:00	0.0094±0.003	0.0116±0.004	0.028	0.0094±0.003	0.0110±0.002	0.01
16:00	0.2553±0.093	0.3717±0.107	0.000	0.2956±0.129	0.3560±0.116	
16:1t9	0.0028±0.001	0.0048±0.002	0.000	0.004±0.002	0.0046±0.002	
16:1c9	0.0100±0.006	0.0210±0.012	0.000	0.0136±0.007	0.0191±0.013	
17:00	0.0073±0.003	0.0094±0.003	0.012	0.0067±0.003	0.0117±0.004	0.000
17:01	0.0037±0.001	0.0045±0.002		0.0039±0.002	0.0040±0.002	
18:00	0.1159±0.035	0.1540±0.030	0.000	0.1287±0.049	0.1584±0.042	0.012
18:1t9	0.0035±0.001	0.004±0.002		0.0050±0.003	0.0038±0.002	
18:1t10	0.003±0.002	0.004±0.002	0.004	0.0038±0.002	0.0039±0.002	
18:1t11	0.0034±0.001	0.0065±0.003	0.000	0.004±0.002	0.007±0.004	0.001
18:1c9	0.1600±0.081	0.2595±0.087	0.000	0.1878±0.107	0.2648±0.122	0.007
18:1c11	0.0148±0.007	0.0228±0.007	0.000	0.0178±0.010	0.0226±0.010	0.043
18:2c9,12	0.3188±0.187	0.5000±0.163	0.000	0.3781±0.243	0.4839±0.184	0.035
18:3c9,12,15	0.0096±0.007	0.0102±0.004		0.0100±0.007	0.0100±0.004	
18:2c9t11	0.0025±0.002	0.0030±0.001		0.0032±0.002	0.0028±0.001	

Serum Fatty Acid Concentrations Pre and Post Meat Consumption Continued

	Beef			Bison		
Serum Fatty acid	**Pre**	**Post**	**p**	**Pre**	**Post**	p
20:4c5,8,11,14	0.0810±0.040	0.1388±0.040	0.000	0.0978±0.053	0.1355±0.051	0.003
20:5c5,8,11,14,14,17	0.0073±0.006	0.0134±0.012	0.028	0.0101±0.012	0.0119±0.014	
22:5c7,10,13,16,19	0.0062±0.003	0.0108±0.005	0.002	0.0076±0.005	0.0098±0.004	0.030
22:6c4,7,10,13,16,19	0.0203±0.011	0.0317±0.015	0.001	0.0254±0.020	0.0303±0.017	
total mg FA	2.184±0.985	3.354±0.870	0.000	2.605±1.372	3.148±0.988	

Difference scores

Serum Fatty acid	**Beef (Pro-Pre)**	**Bison (Pro-Pre)**	**p**
16:1c9	0.002±0.003	0.0046±0.005	0.026
18:2c9,12	0.1811±0.172	0.0001±0.008	0.000

APPENDIX C

STUDY MEAT FATTY ACID COMPOSITION DATA

Weight Percentage of Fatty Acids and Concentrations of Total Fatty Acids in Bison and Beef

	BEEF						BISON					
	Roast		Steak		Burger		Roast		Steak		Burger	
Fatty Acid	Weight %	Concen-tration	Weight %	Concen-tration	Weight %	Concen-tration	Weight %	Concen-tration	Weight %	Concen-tration	Weight %	Concen-tration
14:0	2.84	121.97	2.43	113.00	3.03	455.97	1.28	10.63	1.05	12.33	1.63	119.14
14:1	0.58	26.10	0.41	19.07	0.81	121.67	0.27	1.93	0.09	1.02	0.33	24.29
15:0	0.41	17.07	0.37	16.97	0.5	74.97	0.32	2.87	0.4	4.72	0.6	43.36
15:1	0.1	4.39	0.21	8.72	0.13	19.68	0.26	2.11	0.28	3.32	0.44	32.05
16:0	26.01	1138.64	26.67	1222.43	24.18	3632.22	15.97	135.59	16.87	198.31	17.99	1309.11
16:1t9	0.37	16.41	0.46	21.28	0.42	62.46	0.52	4.02	0.47	5.51	0.66	47.71
16:1c9	3.66	163.24	2.27	105.21	3.23	485.48	1.88	15.81	1.53	17.96	1.47	106.81
16:1c/t11	0.15	6.68	0.07	3.52	0.2	29.53	0	0	0	0	0	0
17:0	1.19	50.77	1.13	52.18	1.27	191.34	1.24	9.83	1.16	13.60	1.37	100.13
17:1	0.93	41.11	0.59	27.39	0.91	137.17	0.7	5.78	0.6	7.03	0.5	36.27
18:0	12.63	557.43	16.04	739.36	13.71	2058.56	22.3	180.38	21.71	254.79	28.25	2057.22
18:1t9	0.11	4.79	0.17	7.91	0.11	16.17	0.46	3.58	0.38	4.44	0.64	46.60
18:1t10	0.19	7.89	0.28	12.67	0.2	30.04	0.56	4.38	0.52	6.06	0.59	43.23
18:1t11	2.25	92.60	3.28	153.06	4.47	670.27	1.81	14.49	1.73	20.31	2.5	182.19

Weight Percentage of Fatty Acids and Concentrations of Total Fatty Acids in Bison and Beef Continued

	BEEF						BISON					
	Roast		Steak		Burger		Roast		Steak		Burger	
Fatty Acid	**Weight %**	**Concen-tration**	**Weight %**	**Concen-tration**	**Weight %**	**Concen-tration**	**Weight %**	**Concen-tration**	**Weight %**	**Concen-tration**	**Weight %**	**Concen-tration**
18:1c9	40.86	1864.10	38.33	1767.14	38.54	5789.55	41.96	321.28	37.96	446.56	32.29	2567.89
18:1c11	1.67	76.85	1.25	57.88	1.53	229.74	1.61	13.32	1.78	20.84	1.18	86.04
18:2c9, 12	2.74	132.01	3.03	138.36	1.69	255.64	5.47	53.64	8.6	101.21	2.17	157.09
18:3c9,12,15	0.16	7.239	0.15	6.94	0.23	33.98	0.65	5.29	0.8	9.45	0.32	22.85
18:2c9t11	0.31	15.25	0.32	15.35	0.44	66.10	0.38	2.76	0.3	3.56	0.3	21.97
18:2t10c12	0	0	0	0	0	0	0	0	0	0	0	0
20:4n6	0.47	25.21	0.29	13.40	0.08	11.43	0.61	7.07	1.31	15.40	0.1	7.05
20:5n3	0	0	0	0	0	0	0	0	0	0	0	0
22:4	0	0	0	0	0	0	0	0	0	0	0	0
22:5n3	0	0	0	0	0	0	0	0	0	0	0	0
22:6n3	0	0	0	0	0	0	0	0	0	0	0	0
Unknown	2.35	101.76	2.27	109.47	4.34	649.34	1.74	18.71	2.45	28.83	3.68	267.60
Total fatty acid		4471.48		4611.30		15021.29		813.48		1175.23		7278.60
Total lipid %		5.35		4.92		17.05		1.57		1.80		8.58

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